CELL CULTURE OF HUMAN CORONARY PLAQUES OBTAINED BY PERCUTANEOUS AND INTRAOPERATIVE ATHERECTOMY

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Cell cultures of human coronary plaque tissue may serve as an in vitro model to study processes contributing to atherogenesis and restenosis. 25/34 patients with coronary artery disease were treated with percutaneous atherectomy (ATH), 9/34 underwent endarterectomy during bypass surgery (OP). We assessed the feasibility to cultivate the removed plaque tissue and thereby compared the results for both approaches. Plaque specimens were cultivated by explant technique or enzymatic disaggregation. More than 90% of the cultured ATH- and OP-cells were identified as smooth muscle cells (SMCs) by their positive staining with monoclonal antibodies specific for SMC α-actin.

Clinical and experimental variables are compared in the two groups (mean±SD):	ATH	OP
Primary lesions	16/25	6/9
Restenotic lesions	9/25	3/9
Number of specimens	4 ± 3	2 ± 1
Total wet weight (mg)	23 ± 16	>400
Success of cultivation	14/25	9/9

Successful cultivation of ATH-SMCs was significantly dependent ($p < 0.001$) on the amount of plaque material submitted. However, it was not related to determinants such as the lesion origin (primary vs restenotic lesions), the age of patients and the stenotic degree pre and post intervention.

Conclusion: Smooth muscle cells are the predominant cell type in symptomatic human coronary plaques. The presented cell culture data confirm the reliability of the percutaneous approach with the prerequisite of an adequate amount of biopsy tissue.

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AMLODIPINE EXERTS A POTENT ANTIMIGRATIONAL EFFECT ON AORTIC SMOOTH MUSCLE CELLS IN CULTURE

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Calcium channel blockers (CCBs) have been shown to impede lesion development in several animal models of atherosclerosis and restenosis following angioplasty; however, their mechanism of action remains unclear. We have used amlodipine in an *in vitro* rabbit aortic explant assay, designed to examine PDGF induced smooth muscle cell (SMC) modulation, migration and proliferation to compare its effects with those of nitrendipine and verapamil.

None of the CCBs had any significant effect on the lag phase prior to outgrowth (modulation) or on the proliferation of SMC. The latter was supported by the inability of CCBs (up to 1μm) to alter ^{3}H-thymidine incorporation into a SMC-line A7r5 (data not shown). SMC migration from the explanted tissue was, however, inhibited by all 3 CCBs. Amlodipine clearly demonstrated greater efficacy than nitrendipine and verapamil over the concentration range studied, inhibiting SMC migration by 35% at concentrations as low as 10pmol/L and did not correlate with their known CCB potency.

(nitrendipine > amlodipine > verapamil in rat aorta)

% Inhibition of PDGF-Induced Migration from Explants

Concentration	1nM	100pM	10pM	1pM	0.1pM
Amlodipine	35.5*	39.0*	35.0*	16.3*	9.8
Nitrendipine	28.5*	13.8*	-	-	-
Verapamil	22.0*	13.5*	-	-	-

- Not tested * $P<0.05$ n=3

In addition, amlodipine, nitrendipine and verapamil were found to inhibit PDGF-induced chemotaxis of the A7r5 SMC line, with amlodipine demonstrating greater inhibition than either nitrendipine or verapamil ($p<0.05$). There was no significant difference between nitrendipine and verapamil ($p>0.1$). In conclusion: (i) Amlodipine, nitrendipine and verapamil do not affect the rate of SMC modulation or proliferation. (ii) Amlodipine clearly demonstrates greater efficacy than nitrendipine and verapamil with respect to antimigrational activity, and is two orders of magnitude more potent. (iii) Inhibition of SMC migration from the media to the intima, and thus reduction in neointima formation, may contribute to the activity of CCB's in animal models of restenosis and atherosclerosis.

ATP-SENSITIVE POTASSIUM CHANNELS MODULATE THE REACTIVE HYPEREMIC RESPONSE

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The mechanisms responsible for reactive hyperemia (RH) following brief coronary occlusions remain unknown. RH may be due, in part, to the opening of ATP-sensitive potassium channels during ischemia which may lower intracellular calcium concentration and, therefore, minimize vasomotor tone. We tested the hypothesis that inhibiting the opening of these channels with a specific blocker, glybenclamide, would reduce the magnitude of RH. In 5 open-chest dogs the circumflex (LC) coronary artery was cannulated and perfused via an extracorporeal circuit. Coronary flow was measured using an in-line electromagnetic flow probe. RH was measured following 30 second LC occlusions performed before and after intracoronary administration of glybenclamide (5 umoles/min for ~30 mins). After glybenclamide, pre-occlusion flow was reduced by 26 ± 3% ($P<.01$), peak RH flow was reduced by 32 ± 7% ($P<.01$) and flow debt repayment was reduced by 80 ± 5 % ($P<.01$). We conclude that blockade of the ATP-sensitive potassium channel profoundly reduces the magnitude of flow debt repayment during RH. This channel may be an important modulator of coronary vasomotor responses following ischemia.

INSTANTANEOUS HYPEREMIC FLOW VERSUS PRESSURE SLOPE INDEX: VALIDATION OF A NEW MEASURE OF VASCULAR RESERVE

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The instantaneous hyperemic flow versus pressure slope index (i-HFVP) is a measure of maximal coronary conductance assessed as the slope of the relation between diastolic hyperemic coronary flow and diastolic aortic pressure, normalized for bed weight. Unlike coronary flow reserve (ratio of hyperemic to basal flow), i-HFVP is independent of changes in mean aortic pressure or basal flow. To compare i-HFVP to microsphere determination of maximal coronary conductance, 18 dogs were studied with high fidelity micromanometers and flow probes. Studies were done in stenotic and non-stenotic states. Radiolabelled microspheres were injected during each of 5 steady-state pressure levels achieved during maximal hyperemia by adjusting an aortic clamp. Maximal hyperemia was maintained by an infusion of adenosine (1mg/kg/min). Microspere-determined maximal coronary conductance was determined from the slope of the linear regression relating microsphere-determined flow values to the 5 mean aortic pressure values. The i-HFVP was calculated using computer generated pressure: flow loops of the electromagnetic flow and aortic pressure tracings digitized at 200HZ. Linear regression analysis of the diastolic portion of this relationship was performed to obtain the slope i.e. the i-HFVP. i-HFVP was most closely related to microsphere determination of subendocardial maximal coronary conductance in both normal beds (10.3 ± 3.9 vs 9.2 ± 5.7 ml/min/mmHgm/100gm, respectively, n.s.) and stenotic beds (3.6 ± 1.6 vs 4.1 ± 2.3, n.s.). Thus, i-HFVP is a novel method of assessing vascular reserve that approximates microsphere determined maximal coronary conductance of the subendocardium.